Blood Products Advisory Committee Meeting
May 13, 2015
Great Room, Building 31
FDA White Oak Campus
10903 New Hampshire Ave.
Silver Spring, MD

Issue Summary

Topic I: Strategies for Implementation of Antibody and Nucleic Acid-based testing for *Babesia microti* in Blood Donors

Issue:

FDA is seeking advice from the Blood Products Advisory Committee (BPAC) on strategies to test blood donors for evidence of *Babesia microti* infection by using licensed tests, when such tests become available. Specifically, the Committee is asked to advise whether antibody testing should be nationally based and year-round and whether NAT should be year-round but limited to certain highly endemic states. In addition, the Committee will be asked to comment on alternative approaches for more limited testing based on geographic risk and seasonal epidemiology in endemic areas, considering the relative risks and benefits of such alternatives.

Background:

Babesiosis, transmitted by a tick-vector, is caused by infections of humans with intraerythrocytic protozoa of the genus *Babesia.* Babesiosis can also be transmitted by transfusion of blood and blood products collected from an infected donor. While babesiosis is transmitted in many parts of the world, including in Europe and Asia, the highest number of clinical and transfusion-transmitted cases of babesiosis are reported in the United States (1, 2). Globally, several species of *Babesia* are attributed to cause infection and clinical diseases; *B. microti* is the dominant species in the U.S. and is responsible for the majority of babesiosis cases (3). Although, babesiosis is prevalent in many parts of the U.S., the vast majority of cases are reported in the Northeastern, mid-Atlantic and upper Midwestern states.

Babesiosis is characterized by a wide spectrum of clinical manifestations that depend on the host age, and its immunological and health status. In children and young healthy adults, *B. microti* infection leads to asymptomatic or mild disease. However, disease may be severe and even fatal in neonates, the elderly and the immuno-compromised, including cancer patients, with fatality rates of 5-20% reported (1, 3).

The majority of *B. microti* infections are asymptomatic and never diagnosed (1). While the precise duration of persistence of *B. microti* infections in healthy adults is not known, in limited studies the parasitemic period is reported to last from 2-7 months (4). According to one report, infections (based on polymerase chain reaction (PCR) results) may persist for up to 27 months without overt clinical illness (5). Although *Babesia* transmission is seasonal and coincides with tick activity (traditionally May-September), both tick-borne and transfusion-transmitted infections are reported year round resulting from chronic, unresolved parasitemia. The proportion of *Babesia* infections that persist as asymptomatic, chronic infections is not known. In addition, in endemic areas, the contribution of reinfection to chronic parasitemia has not been investigated. In the absence of donor screening, prospective donors with asymptomatic infection are difficult to recognize and defer, and blood and blood components collected from such donors may result in transfusion-transmitted babesiosis (TTB), leading to potentially fatal clinical illness.

Currently, *B. microti* is the highest-ranking pathogen that is transmitted by blood transfusion in the U.S. for which no donor screening is available (2). Data from the national babesiosis surveillance program and annually

reported TTB cases show that the TTB-risk to U.S. blood supply is increasing. In recognition of this growing threat, the AABB Transfusion Transmitted Diseases (TTD) sub-committee has identified transfusion-transmitted *Babesia* infections as having one of the highest priorities among emerging infectious diseases that pose a risk to blood safety (6). In addition, given the growing public health concern, in 2011, babesiosis became a notifiable disease and in 2013, 29 states participated in the national babesiosis surveillance program. Since 2008, FDA has addressed the issue of transfusion-transmitted babesiosis in public settings (7), including at a 2010 BPAC meeting (**http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/BloodProductsAdvisoryCommittee/UCM225388.pdf**). At the 2010 BPAC meeting, the Committee was requested to advise FDA on an appropriate testing strategy to mitigate the TTB risk in blood transfusion recipients, including the concept of a regional testing strategy. In addition, FDA also sought the Committee's comments on the suitability of donor screening either by a nucleic acid-based test (NAT), an antibody test, or both, given the current technology limitations. Based on the information available at that time, the Committee recommended the concept of selective, regional testing of blood donors for *Babesia*. The Committee did not provide advice on the question of most suitable technologies for donor screening for *Babesia*.

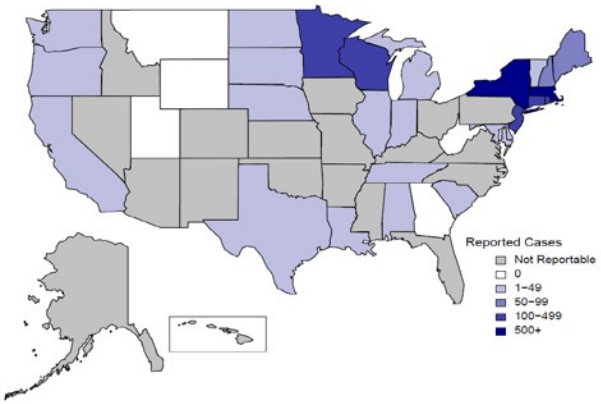
At the current BPAC meeting, FDA plans to discuss the following: (1) Information on the clinical babesiosis and TTB cases by state; (2) Monthly distribution of *Babesia*-infections by state; (3) Outcome of clinical studies for donor screening for *B. microti* by two manufacturers using investigational tests ; and 4) FDA risk assessment analysis on nation-wide babesiosis risk based on the babesiosis cases reported to the Centers for Disease Control (CDC) and the Centers for Medicare and Medicaid Services (CMS) datasets.

The Committee will be asked to consider this information and to advise FDA on the most appropriate strategies to implement donor screening for *B. microti* to mitigate the TTB risk in blood transfusion recipients by using licensed tests, including whether antibody testing should be nationally based and year-round and whether NAT should be year-round but limited to the five highest *B. microti*-risk states.

Epidemiology of *Babesia microti* in the United States:

Clinical Cases of Babesiosis as Reported under the National Surveillance Program: In the recent years, the number of tick-borne infections and their geographic distribution has increased (2, 8). In 2011, national surveillance for babesiosis began in 25 jurisdictions (24 states and New York City) and a total of 1,128 confirmed and probable cases were reported to CDC (9) . Since then, the number of states where babesiosis is reportable is steadily increasing. In 2012, babesiosis was reportable in 24 states; 937 cases were reported in 15 states and no cases were reported in 9 states (10). In 2013, the disease was reportable in 29 states and 1,792 cases were reported (11). While cases of babesiosis were reported in all months, in the majority of cases (about 85%), onset of symptoms were reported during the months of June-August. It is important to note that babesiosis is still not reportable in all states, including in states where clinical babesiosis and/or TTB cases are reported to occur (e.g., Florida, Georgia and Pennsylvania). Overall, since the inception of national surveillance in 2011, babesiosis cases were observed in 26 states which excludes several *Babesia*-risk states because disease is not reportable in those states (Figure 1). Thus, *Babesia*-risk is widely prevalent across the U.S. due to expanding areas of transmission and infections acquired during travel to endemic areas (2).

Figure 1: State-Level Distribution of Babesiosis Cases, as Reported to CDC, 2011-2013

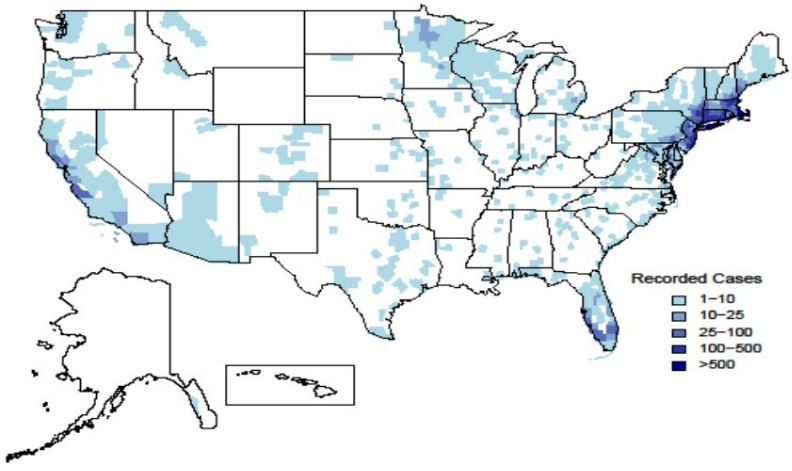


Sources: Refs. 9-11; http://www.cdc.gov/mmwr/mmwr_nd/; http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6332a6.htm

Babesiosis Cases Among the Elderly as Recorded in Large Medicare Databases: As elderly persons are at a higher risk for clinical babesiosis with severe complications (2) and published literature suggests the disproportionately high utilization of blood in this population (8), the elderly are more likely to be diagnosed with babesiosis and be at an increased risk for TTB. **Since *B. microti* infections are less likely to remain asymptomatic in the elderly, the babesiosis rate in this population group may be a sensitive marker for modeling TTB risk in the U.S. blood donors.** The Centers for Medicare & Medicaid Services (CMS) administers Medicare, a national health insurance that provides coverage to virtually all U.S. elderly persons ages 65 and older, and maintains large administrative databases (12). A retrospective population-based study assessed babesiosis occurrence among elderly Medicare beneficiaries in the United States during 2006-2013 by year, age, gender, race, state of residence, and diagnosis months.

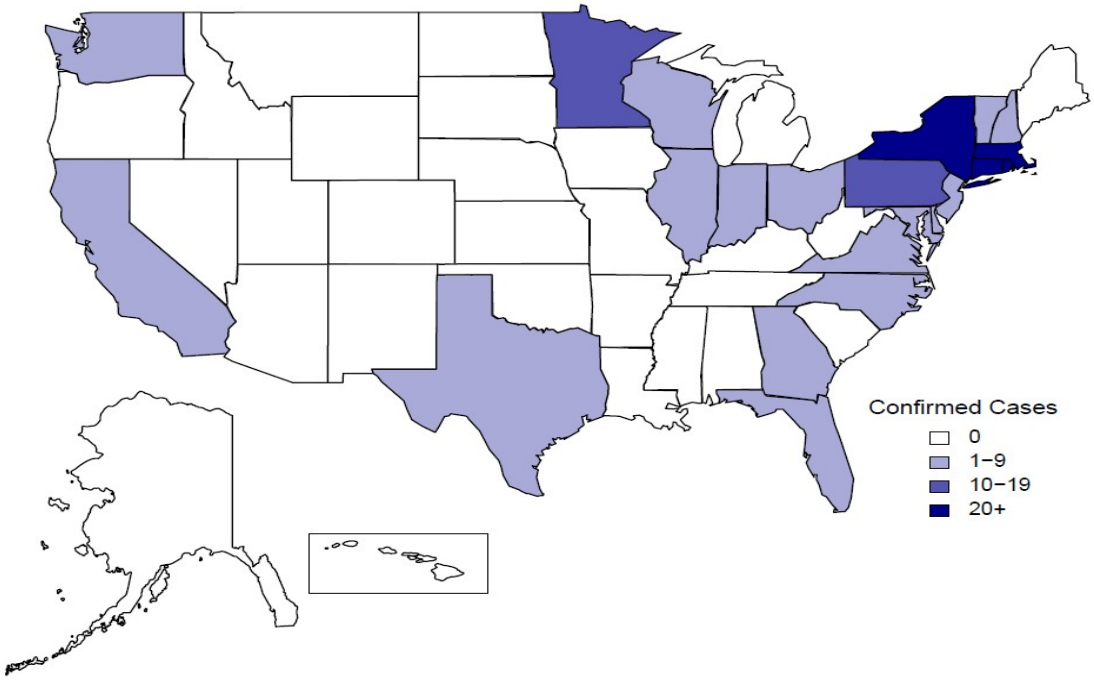
A total of 10,301 elderly Medicare beneficiaries had a recorded babesiosis diagnosis during 2006-2013, an overall rate of about 5 per 100,000 persons. The results showed a significant increase in babesiosis occurrence over time ($p<0.05$), with the largest number of cases recorded in 2013 (N=1,848) and the highest overall babesiosis rates (per 100,000) in five Northeastern states: Connecticut (46), Massachusetts (45), Rhode Island (42), New York (27), and New Jersey (14) (Figure 2). About 75% of all babesiosis cases were diagnosed in the months of May through October.

Figure 2: Babesiosis Occurrence among Medicare Beneficiaries during 2006-2013



Transfusion-Transmitted Babesiosis: In the absence of any nationwide epidemiological studies, our knowledge on TTB-risk in U.S. is based on the cases reported to CDC and FDA by the State Health Departments and blood establishments (Figure 3). Since 1980 when the first U.S. case of TTB was reported (13), more than 200 cases of transfusion-associated infections have been documented (8-11, 14) in 22 states, although the actual number of cases are thought to be much higher. Importantly, in recent years, there has been a marked increase in the TTB cases; during the 34 years of reporting about 28% of all cases were recorded during the period of 2010- 2014. Importantly, TTB is responsible for the highest percentage (38%) of transfusion-related infectious fatalities reported to the FDA in transfusion recipients (4). It is also noteworthy that the number of states where TTB cases are occurring is also expanding; no cases were reported from Georgia and Illinois prior to 2010. TTB cases outside of *Babesia*-endemic states are attributed to travel to endemic states, blood donations in non- endemic states by donors who had acquired their infections elsewhere, and shipment of infected blood from endemic to non-endemic states.

Figure 3: State-Level Distribution of Transfusion-Transmitted Babesiosis Cases, 1979-2014



Sources: Refs. 8, 14

Transfusion-transmitted infections cause high fatality in transfusion-recipients; from 1979-2009 about 28 deaths are attributed to complications from TTB (8). Additionally, four TTB-associated deaths were reported to FDA between 2010-2014 (14).

Recently antibody and NAT-based assays have been used to conduct limited epidemiological surveys in *Babesia*-endemic states. These studies have revealed important information about the percentage of prospective asymptomatic donors, the relationship between the sero-positivity and parasitemia, and seasonality of transmission in endemic areas. In a seroprevalence study conducted from July through September by the American Red Cross (ARC) in endemic and non-endemic areas in Connecticut, 30 (0.9%) of 3,490 donations were positive for IgG antibodies to *B. microti* by immunofluorescent assay (IFA). Importantly, 10 (53%) of 19 antibody-positive donors were also positive by PCR indicating that a proportion of antibody-positives are parasitemic (15). In another eight-year (2000-2007) seroprevalence study in Connecticut and Massachusetts,

blood samples were collected every other month during the year. Among the 23,304 donations included in the study, 267 (1.14%) were seropositive by IFA. The yearly aggregate seroprevalence rate was relatively stable over the eight-year study period. Although the highest seroprevalence was observed from July through September, seropositive donors were identified in every month of the year (16). In a recently published longitudinal study, 18 of 84 (21%) enrolled seropositive donors had evidence of parasitemia in samples collected during a three year period (17).

Data collected by the ARC Hemovigilance Program suggested that, of 17 antibody-positive donors implicated in causing TTB, 11 (65%) were residents in *Babesia*-endemic areas while four (24%) were from non-endemic areas who had a history of travel to endemic areas (18). This gives credence to the notion that the risk of transmitting *Babesia* infection through blood transfusion is not limited to donors living in endemic areas.

Antibody as Surrogate of B. microti Infection: Antibodies are a reliable marker of exposure to *B. microti* parasites; in earlier studies, about 20% of sero-positive individuals were also found be parasitemic as determined by a PCR test (17, 19). However, due to a lack of knowledge on minimum parasite burden in chronic asymptomatic infections, limitations in the volume of blood that can be used to prepare genetic material and the sensitivity of NAT used for detection, it is difficult to ascertain the ability to transmit *B. microti* infections by sero-positive, but PCR-negative blood units. While the *B. microti*-specific antibodies may persist for several years, in limited studies, clearance of parasitemia (as measured by microscopy and PCR) is often associated with decline in antibody titers. Ruebush et al. determined the development and persistence of *B. microti* antibodies in 16 patients between one week and year after the onset of illness. The authors reported that all 16 patients developed IFA titers of 1:1,024 or 1:4,096 between the first 3 to 4 weeks after clinical symptoms. The antibody titers began to decline in the next 2 to 3 months and ranged between 1:16 to 1:256 at 5-7 months after onset of illness and were maintained at that level for up to 13 months (20). In another prospective study, *B. microti*-infected individuals from southeastern Connecticut and Block Island, Rhode Island were followed for up to 27 months to detect the episodes of illness and evidence of parasitemia (by microscopy and PCR) and sero-conversion. The authors reported a strong relationship between the presence of *B. microti* PCR-amplifiable DNA and persistence of IFA IgG titers. In 12 patients who were monitored for babesial DNA and persistence of antibody, the circulating DNA lasted for 3 months or more after the initial diagnosis which also paralleled the rise and decline of antibody titers. At 12 months after the initial diagnoses, antibody levels either returned to baseline or dropped from a peak reciprocal titer of 1:1400 to 1:200 (5). Recently, in a longitudinal study, investigators assessed the course of *B. microti* infection in sero-positive donors; 6 donors had become sero-negative within 6-9 months of being parasitemic. On the other hand, 3 donors remained sero-positive over three years of follow up period, despite having received anti-babesial treatment (17). These results suggest that while in a subset of individuals, anti-*B. microti* antibody response may persist for several years, the majority of donors experience a sharp decline in antibody titers after the initial infection. The prolonged antibody levels in *B. microti* exposed individuals may be attributed to protracted asymptomatic infections, reinfections or recrudescence due to compromised immune status (5). Results from the long-term follow up studies in a larger cohort of donors conducted under IND protocols would provide in depth information on duration of persistence of *B. microti*-specific antibodies in residents from endemic and non-endemic areas.

Donor Testing for B. microti by Investigational Tests: Recently, two manufacturers – IMUGEN (PCR and IFA tests) and Immunetics enzyme immunoassay (EIA) have publicly released the results of donor screening for *B. microti* using their investigational tests (19, 21-23). In the Immunetics study, the reported results showed the following percentages of EIA repeat reactive (RR) in 15, 000 (three groups of 5,000) un-linked donors from three distinct geographic areas: 0.92% (Suffolk Co., NY: high endemic); 0.54% (New York City, NY: low endemic) and 0.16% (Arizona: non-endemic) (22). Only one EIA repeat reactive (RR) from the high *Babesia*-

endemic area was also PCR positive (22) . In the IMUGEN study conducted by the American Red Cross, 386 of 102,027 donors were RR by a combination of PCR and IFA tests. Of these 74 were both PCR and IFA positive while 9 were PCR positive and IFA negative demonstrating the presence of window period cases (time for the first appearance of parasitemia and seroconversion) in the donor populations (Table 1). The percentage of *Babesia* IFA and/or PCR positive donors was highly diverse in endemic states - Massachusetts and Connecticut (high endemic): 0.46% (8 window units); Minnesota and Wisconsin (low endemic): 0.11% (1 window unit). The percentage of *Babesia* IFA positive donors in non-endemic states –Arizona and Oklahoma was only 0.025% while no PCR positive donor was identified in these states. While the majority of PCR and IFA positive donors were identified during the high transmission months, a substantial number of IFA positive donors were identified year round while PCR positive donors were detected in all months except in the month of April (19).

Table 1. Summary of *Babesia microti* IFA and/or PCR Reactive Blood Donors in the IMUGEN IND study

Blood donors who are IFA or PCR–reactive	386 (100%)
Blood donors who are IFA reactive but PCR-negative	303/386 (78.5%)
Blood donors who are PCR positive among all reactive donors (parasitemic donors)	83/386 (21.5%)
Blood donors who are PCR-reactive but IFA negative (window cases)	9/386 (2.3%)
PCR-reactive but IFA negative blood donors who seroconverted in follow up study	8/9 (89.0%)

Source: Refs. 19, 23

Benefit-Risk Assessment of *Babesia* Infection in the United States Blood Donors:

We developed a benefit-risk assessment model to estimate: 1) the current potential risk of babesiosis in U.S. blood donors; 2) the potential reduction in TTB risk under various testing strategies; 3) the potential blood unit loss due to false positive screening test results under the same testing strategies, and 4) the positive predictive value (PPV) of testing. The testing strategies explored the effect of antibody-only testing as well as more extensive testing with both antibody and NAT in selected states or throughout the U.S. In one approach testing focused on the states with the highest prevalence of babesiosis, since this would yield the greatest reduction in TTB risk for the fewest donors to be tested and the highest PPV. We also modeled an approach that focused on states with the highest number of estimated donors with babesiosis.

We based the model primarily on estimated babesiosis rates (per 100,000 elderly Medicare beneficiaries) by state using Center for Medicare and Medicaid Services (CMS) data for the years 2006-2013 (See Appendix for Study Design and Methods). CMS is an extremely large data set covering millions of beneficiaries aged 65 years and older in the U.S. The size and geographical coverage of the CMS data set provides the statistical power needed to estimate rates of a diversely prevalent disease like babesiosis by state. Specifically, the number TTB units identified and interdicted based on the sensitivity of testing strategies and donor blood loss due to false positive testing results were estimated using formulas (See Appendix for Study Design and Methods) that combined data on: 1) babesiosis risk by state, 2) blood donation rate, 3) average number of times a donor donates annually, 4) the sensitivity and specificity of the hypothetical screening tests, 5) the time before NAT

can detect *B. microti* parasites (window period), and 6) the time from NAT positive test result to seroconversion. Due to the limitations in data availability, there is substantial uncertainty about several of the inputs used in the TTB risk model. These uncertainties include: 1) the sensitivity and specificity of the tests that may eventually be approved, 2) the limit of detection by NAT-based tests that may be approved, 3) the time to earliest parasite detection by NAT and progression of the disease in humans from the moment of infection until the development of detectable antibodies, and 4) the infectivity of a unit of blood collected from an infected donor at different stages of the infection. We believe that the benefit-risk model provides helpful insight to assess the babesiosis-risk by states and reduction in TTB-risk by implementation of various testing strategies that support decision-making, but we recognize the limitations discussed above.

The estimated babesiosis occurrence trends from CMS data among the elderly are highly correlated with CDC data on reported cases of clinical babesiosis by year, state, and month of diagnosis (Figures 4-6). However, the estimated CMS babesiosis rates in the elderly are much higher than the CDC babesiosis occurrence rates in the general population since *B. microti* infections are less likely to remain asymptomatic in individuals who are 65 years or older as compared to general population, and because of potential underreporting of cases to CDC. Therefore, we based our estimates on babesiosis occurrence rates in the U.S. elderly.

Assessment of Babesiosis Risk in the United States using CMS and CDC data:

Babesiosis Cases and Rates in CMS Datasets: During the 8-year period (2006-2013), we identified 10,301 unique U.S. elderly Medicare beneficiaries with recorded diagnosis for babesiosis. Babesiosis occurrence varied by year, diagnosis months, and state of residence. The highest babesiosis rates occurred in June, July, and August (trends similar to CDC results), with 79% of all cases diagnosed from April through October and with 14.6% of all cases diagnosed from December through March (Figures 4-5).

Figure 4: Babesiosis Cases (Gray Bars) and Rates (Black Line) by Year among Elderly Medicare Beneficiaries, United States, 2006-2013.

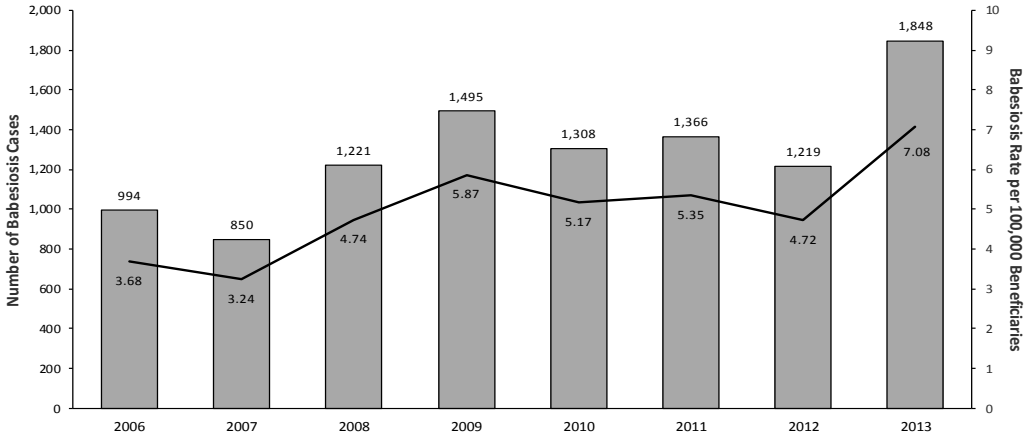
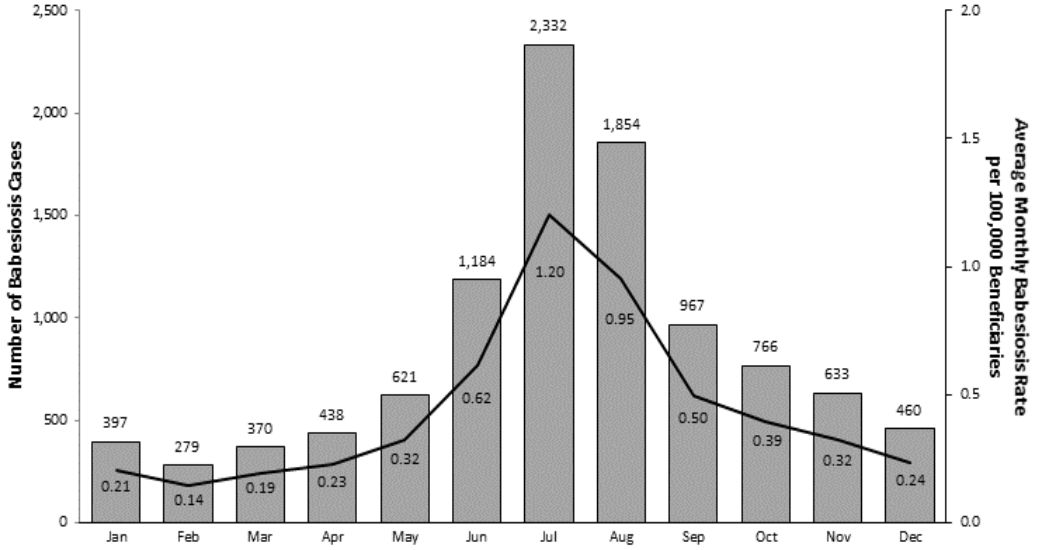


Figure 5: Babesiosis Cases (Gray Bars) and Average Rates (Black Line) by Month of Diagnosis among Elderly Medicare Beneficiaries, 2006-2013.



Both CMS and CDC data show that most of the cases are in the Northeast and upper Mid-west regions of the U.S. The CMS data shows cases in many more areas of the U.S. than the cases reported to CDC (Figures 1 and 3).

The overall national babesiosis rate was 5 per 100,000 elderly Medicare beneficiaries. Some states had rates about 10 times higher than the national rate. All states, except Wyoming, had at least one babesiosis diagnosis recorded among the U.S. elderly during 2006-2013. The highest overall babesiosis occurrence rates (per 100,000) were in five Northeastern states: Connecticut (46), Massachusetts (45), Rhode Island (42), New York (27), and New Jersey (14). The top five babesiosis-endemic states accounted for 76.6% of all cases identified in the U.S. elderly. Other states also had babesiosis recorded including, Maryland (7), Virginia (4), Pennsylvania (3), Florida (3), and California (2). Connecticut through California (16 states plus the District of Columbia (DC)) accounted for 95.3% of all babesiosis cases (Figure 6 and Supplementary Table 1).

Figure 6: Comparison of CMS (2006-2013) and CDC (2011-2013) Estimates of Babesiosis Rates by State.

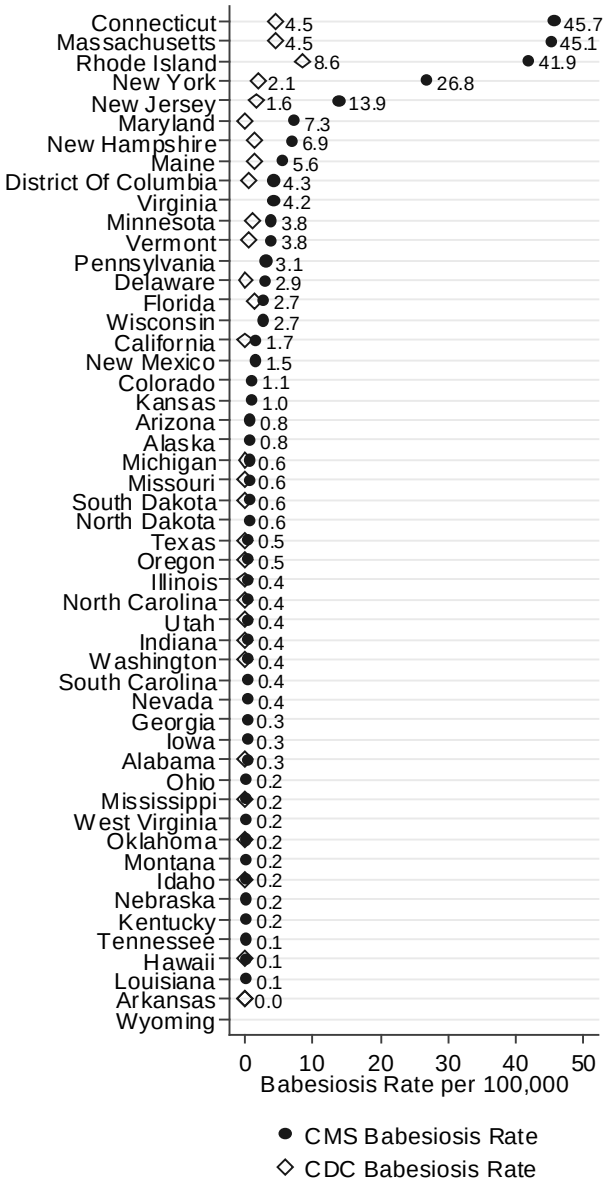


Figure Legend: The double dot plot compares the babesiosis rate per 100,000 persons by state derived from CMS and CDC data. States are ordered by the babesiosis rate estimated from CMS data with the highest rates at the top of the plot. Solid circles represent the CMS babesiosis rate and hollow diamonds represent the CDC babesiosis rate. The CMS babesiosis are higher than the CDC rates, however they follow a similar pattern across the states.

Babesiosis Cases and Rates in CDC and Comparison to CMS Data: The CDC has reported clinical cases and TTB cases with corresponding estimated babesiosis rates by state (Supplementary Table 2 and 3). Using CDC data from 2011 to 2013, the highest overall babesiosis rates (per 100,000) were identified in five Northeastern states: Rhode Island (8.6), Connecticut (4.5), Massachusetts (4.5), New York (2.1), and New Jersey (1.6). The top five babesiosis-endemic states accounted for 85.1% of the 3,862 cases reported to CDC during 2011-2013. Herwaldt and colleagues (Supplementary Table 3) identified TTB cases with corresponding rates by state. During 2000-2009, a total of 109 TTB cases were reported in 18 states. The five babesiosis-endemic states (Rhode Island, Massachusetts, Connecticut, New York, and New Jersey) accounted for 74.3% of the 109 TTB cases.

Overall, there is a strong concordance in the ranking of states based on babesiosis rates (Supplementary Table 4). The rankings are similar for CMS and CDC (clinical cases and TTB cases) data, especially for endemic states. Babesiosis occurrence trends over time and by month of diagnosis using CMS data are also in agreement with the results from CDC on babesiosis reporting. However, babesiosis occurrence identified using CDC case reporting data was substantially lower than the babesiosis occurrence identified by CMS. This difference could be due to a high underreporting of babesiosis in the general population since babesiosis is more likely to be asymptomatic in younger individuals. Given the strengths and limitations of CDC and CMS data, we believe that babesiosis rates based on CMS data among the U.S. elderly provides the most useful available population-based estimate of babesiosis occurrence for the purposes of this benefit-risk assessment.

Results of TTB Benefit-Risk Assessment: We used the babesiosis rates in the elderly, based on CMS datasets, to estimate the number of TTB-risk units prevented, the percent risk reduction from baseline, and the number of false positive results, and the PPV overall and by state, for the one-test (antibody alone) and two-test (NAT and antibody) blood donor screening strategies (See Appendix for Study Design and Methods). In order to understand the impact of inclusion/exclusion of individual states on babesiosis risk-reduction by testing, we estimate the endpoints by starting from the *status quo* baseline of no testing and adding testing requirements in additional states, one at a time. We used two possible sorting orders for adding states. The first added testing requirements in states based on the state's babesiosis rate. The second added testing requirements based on the estimated number of donors with babesiosis—babesiosis rate x population x donation rate—in the state. We also discuss the results of eight selected testing scenarios (Table 2).

The model estimates that 601 blood donors per year develop babesiosis and that those donors donate 1,022 units. Year-round, national serology antibody testing is estimated to reduce risk by a little more than 90% with a PPV of about 20% (Supplemental Figure 1a and 1b). The projected number of donors with babesiosis detected by state with national antibody testing, under our baseline assumptions, ranges from zero to 202 donors (Figure 7). This translates into a reduction of zero to 343 blood units prevented from entering the blood supply by state (Supplementary Table 5 and 6). The projected risk reduction would fall to 89.5% if the window period before seroconversion is 28 days instead of 21 days as modeled. Testing year-round in the states with the five highest babesiosis rates—Connecticut, Massachusetts, Rhode Island, New York, and New Jersey—would reduce TTB risk by about 70%. Testing year-round in the states with the five highest estimated numbers of donors with babesiosis— New York, Massachusetts, Connecticut, New Jersey, and California—would reduce TTB risk by about 73%. Using either ranking (babesiosis rates or numbers of donors with babesiosis), 27 states would need to use antibody testing year-round to reach a 90% risk reduction.

Continuing with national year-round antibody testing and adding NAT in the highest endemic states or nation-wide will further reduce the TTB risk. The NAT would detect the parasites during some phase of the window period prior to seroconversion, and therefore would be more effective than antibody testing alone. Using antibody testing and NAT nationwide testing, year-round would approximately reduce the risk of TTB by an additional five percentage points (Supplemental Figure 2a and 2b). Adding year-round NAT testing in the states with the five highest babesiosis rates would reduce the TTB risk by about 3.6 percentage points above national year-round antibody testing. NAT in the states with the five highest babesiosis rates during the months of April through October would also reduce the TTB risk by about 2.5 percentage points above national year-round antibody testing.

The PPV improves as additional states implement NAT, because we are assuming that antibody and NAT plus antibody each have specificity of 99.98%. Consequently, a few more true positives are detected without detecting any more false positive results. Supplemental Figure 2a and 2b illustrates the effect on overall PPV as NAT is added to antibody testing in successive states at decreasing *Babesia* risk. After adding the state of Virginia, the PPV plot follows a step pattern. The PPV remains the same for several states added and then steps

up to a slightly higher PPV. This occurs because the estimated number of additional positive donors remains constant for the addition of several states before increasing by one donor. Under our baseline assumptions, about 2,400 donors would have false positive results, but this estimate is very sensitive to the specificity of the tests applied. If the specificity is reduced from 99.98% to 99.95% the number of donors with false positive results would increase to more than 6,000. PPV falls rapidly if specificity is not close to 100% (Figure 8).

The estimates presented here assume that a blood unit from an infected donor has a 100% probability of causing TTB, if it is transfused into a recipient. There are no human data with which to accurately calculate the true probability whether a unit of blood from an infected donor would cause TTB. For the sensitivity analysis, we used an alternative assumption that blood units were much less infectious during the early infection period in which NAT cannot detect the parasites. The analysis showed similar percentage risk reduction.

Table 2 summarizes the TTB risk reduction, PPV, units from positive donors interdicted, and donors lost due to false positive test results under a few selected testing scenarios as calculated by the FDA risk assessment model. As anticipated, an incremental TTB risk reduction is achieved when year-round antibody testing is expanded to additional states: 5 states − 70.1% risk reduction or 716 positive blood units interdicted; 16 states plus DC − 87.8% risk reduction or 897 positive blood units interdicted; 50 states plus DC − 91.4% risk reduction or 939 positive blood units interdicted. Year-round NAT testing along with year round antibody testing had a further beneficial effect on the TTB risk reduction: 5 states − 73.7% risk reduction or 752 positive blood units interdicted; 16 states plus DC − 92.3% risk reduction or 943 positive blood units interdicted. In scenarios where year-round antibody testing plus NAT testing had to be implemented in 50 states plus DC, or antibody testing in 50 states plus DC and antibody and NAT in the 16 states, the benefits of testing would remain unchanged: 96.0% risk reduction or 985 positive blood units interdicted. In comparison, antibody testing in 50 states plus DC and NAT testing along with antibody testing in 5 states would reduce the TTB risk by 95.0% or 975 positive units interdicted. Incorporation of NAT testing along with antibody testing in 50 states plus DC under any testing scenario did not influence the number of false positive units while enhancing the benefits of TTB risk reduction (Table 2). There was a large shift in the number of false positive donors when antibody and NAT testing was expanded from 16 states plus DC to 50 states plus DC (1,093 false positive donors versus 2,422 false positive donors) reflecting the much larger test population. Since the average number of donations per year is about 1.7, the number of units lost due to false positive test results would be about 70% higher—1,860 in the 16 states plus DC scenario and 4,120 in the 50 states plus DC scenario. In addition, PPV improved significantly if antibody and NAT testing was limited to 16 states plus DC instead of 50 states plus DC (33.6 PPV versus 19.1 PPV).

Overall, the FDA risk model suggests that antibody testing in 50 states plus DC and antibody plus NAT testing in the 5 highest risk states would provide the highest TTB risk reduction with the least number of states where both antibody and NAT testing would have to be implemented.

Table 2. Summary of TTB Benefits and Risks Under Selected Testing Scenarios

Testing Scenario	Percent TTB Risk Reduction	Positive Predictive Value	Units From Positive Donors Interdicted	False Positive Donor Test Results
No Donor Testing	0	0	0	0
Antibody Only, 5 States	70.1	57.1	716	315
Antibody and NAT, 5 States	73.7	58.3	752	315
Antibody Only, 16 States plus DC	87.8	32.5	897	1093
Antibody and NAT, 16 States plus DC	92.3	33.6	943	1093
Antibody Only, 50 States plus DC	91.4	18.6	939	2422
Antibody in 50 States plus DC, NAT in 5 States	95.0	19.1	975	2422
Antibody in 50 States plus DC, NAT in 16 States plus DC	96.0	19.3	985	2422
Antibody and NAT in 50 States plus DC	96.0	19.3	985	2422

Figure 7: Double Dot Plot of the Projected Number of Positive Units Detected in Each State and the Cumulative Percentage of Risk Reduction Using Antibody Tests in Selected States.

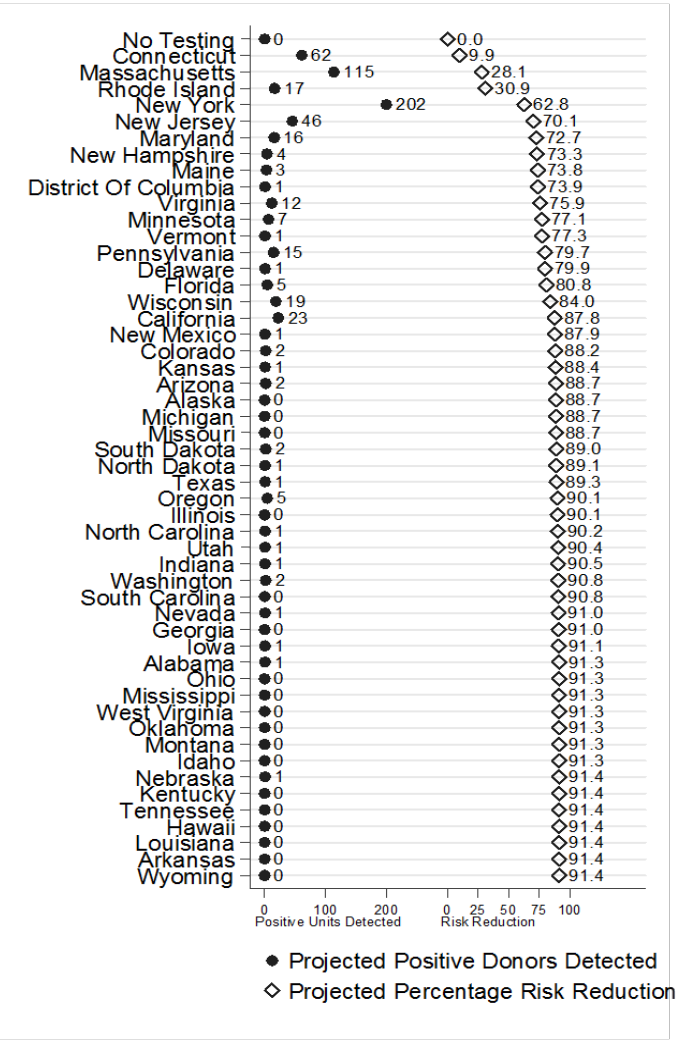


Figure Legend: We assume a 21 day window period for the antibody test and a sensitivity of 97% after the window period.

Figure 8: Relationship Between Specificity of Babesiosis Tests and PPV

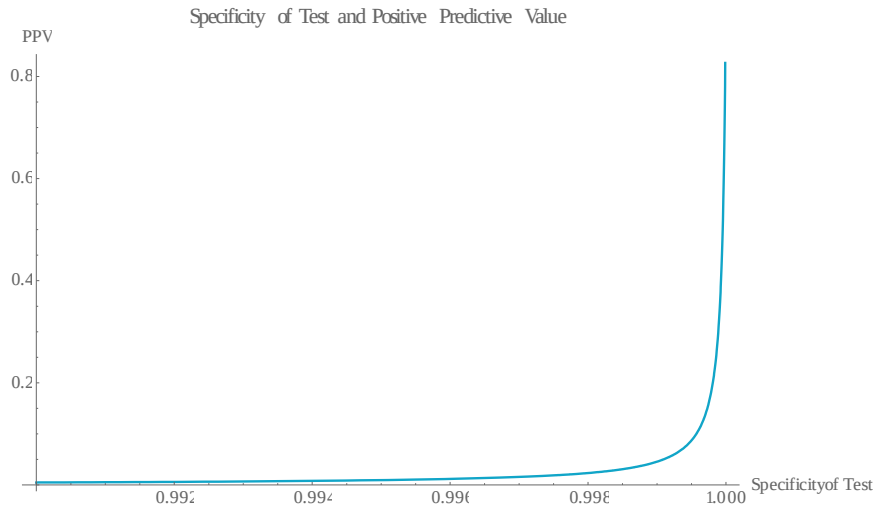


Figure Legend: The plot in Figure 8 shows the relationship between the specificity of a hypothetical babesiosis test and the PPV. The PPV falls rapidly if the specificity is not near 100%.

Discussion:

Possible Strategies for Babesia Screening in Blood Donors Using Licensed Tests: There are significant geographical disparities in risk of natural *Babesia* infection in the U.S. and a majority of *Babesia*-risk (babesiosis cases and TTB) cases are localized in the Northeastern and Upper Midwestern states. However, clinical babesiosis cases are reported from 26 states (CDC data) while TTB cases have occurred in 22 states. Furthermore, CMS beneficiary claims with a recorded babesiosis diagnosis were filed from all states (including the District of Columbia) with the exception of Wyoming (Figures 2 and 6). These results clearly demonstrate that actual TTB cases and *B. microti*-infections in prospective blood donors routinely occur outside of natural transmission areas. It is noteworthy that the reported babesiosis cases and TTB cases represent only a fraction of actual *B. microti*-risk because 1) the majority of babesiosis cases are misdiagnosed or undiagnosed; 2) so far (through 2013), only 31 states participated in the *Babesia* surveillance program, leading to non-reporting of identified cases in the states that do not participate in the national program to CDC or to other public health authorities. In addition, CDC and CMS datasets suggest that the nation-wide risk of *B. microti* infection is higher than previously thought and the number of actual cases and the areas of parasite transmission are increasing. Nonetheless, in the absence of nation-wide epidemiological studies to assess the risk of *Babesia* infection in U.S., there is a lack of accurate knowledge on *Babesia*-risk especially in areas where natural transmission is not known to occur. Together, these factors suggest that to fully mitigate the *B. microti*-risk in the blood supply, nation-wide antibody screening would be the most suitable strategy.

The TTB benefit-risk assessment indicates that a national, year-round antibody testing strategy would reduce the TTB risk by 90% or more under reasonable assumptions about the window period and sensitivity of the test. Assuming a test with a specificity of 99.98%, the PPV will be about 20% and about 2,400 donors (4,120 units) will receive false positive results nation-wide.

Although tick-borne *B. microti* transmission in endemic areas is seasonal and mostly occur during the months of May-September (9-11), both clinical cases and TTB cases are reported in all months of the year (6). Likewise, epidemiological studies in endemic areas have reported the presence of sero-positive and parasitemic donors year round. Additionally, recently infected parasitemic, but antibody negative donors in areas of high endemicity present a risk to blood safety. Indeed, 9 NAT-positive but IFA negative donors were detected in endemic areas in the IMUGEN IND study (19). These data indicate the value of NAT testing, in addition to antibody testing, is high in the highest *Babesia*-risk areas.

Instituting NAT testing in the top five highly endemic states (Connecticut, Massachusetts, Rhode Island, New York and New Jersey) year-round in addition to antibody testing in all states plus DC is estimated to reduce TTB risk by 95%, in the best case scenario, when compared to the current no testing scenario. The actual rate of reduction may be lower if there is a window period before NAT can detect the parasite or if the test sensitivity is less than what was assumed in the risk assessment. We estimate that about 78% of cases that are detectable by NAT but not by antibody will be prevented if antibody and NAT are implemented in those top five *B. microti* endemic states and antibody only is instituted in all other states. Finally, testing the top five babesiosis-endemic states throughout the year with both antibody and NAT and all other states with only antibody throughout the year is likely to prevent similar number of TTB risk units as compared to antibody only throughout the year with NAT applied seasonally from April thru October in all states. Thus, in order to detect the maximum number of window cases, NAT testing, in conjunction with antibody tests, appears to be a prudent strategy in the five highest endemic states. A wider application of NAT testing may be considered based on incremental benefits in risk reduction by state as shown in Supplemental Figure 1a and 1b.

Given the current knowledge on *B. microti*-risk in the U.S. and the status of *Babesia*-NAT and antibody-based investigational tests under development (19, 21-23), FDA is proposing the following testing strategy at such time that licensed tests may become available:

***Year-round nation-wide testing by antibody-based tests*:** Based on the FDA risk analysis utilizing the data inputs of clinical babesiosis cases (CDC and CMS data) and TTB cases (CDC and FDA data), highly sensitive and specific antibody-based licensed tests might be used for nation-wide, year round testing of blood donors for *Babesia*-risk.

***Year-round testing by NAT in the highest risk region in addition to antibody-based tests*:** Based on the FDA risk analysis, a combination of highly sensitive and specific antibody and NAT-based licensed tests might be used for donor screening for *Babesia*-risk year round in the following top five *B. microti*-endemic states: Connecticut, Massachusetts, Rhode Island, New York and New Jersey. Year-round rather than seasonal NAT in the highest risk states is proposed to minimize the complexity of stopping and restarting testing for the period between the months of active tick-biting.

Questions for the Committee:

1. Do the scientific data support the concept of nation-wide, year round testing of blood donors for *Babesia*-risk by an antibody-based test?
    b. If not, please comment on alternative options that FDA should consider, including limitation of antibody testing to specific states.
2. Does the Committee agree that, based on the risk analysis, both NAT-based testing should be performed year round in addition to year-round antibody testing in blood donors in the five highest endemic states?
    b. If not, please comment on alternative options that FDA should consider, including 1) limiting use

of NAT to the tick-bite season in five states, and 2) wider geographical use of NAT year round or limited to the tick-bite season.

3. Please comment whether it would be appropriate to apply a time-based deferral for those donors who had *B. microti*-positive test result(s)?
    b. If so, please advise on a suitable deferral period for donors who had *B. microti*-positive test result(s)?

Appendix:
Additional Information about Methods and Supplementary Tables

Study Design and Methods Details:

We used large Medicare databases to assess babesiosis incidence among elderly Medicare beneficiaries aged 65 years or older during the calendar years 2006-2013. We included beneficiaries who were continuously enrolled in Medicare fee-for-service Parts A and B for at least 365 consecutive days prior to his or her last month of enrollment in that year. We excluded beneficiaries enrolled in Medicare Part C at any point in this 365-day period. Babesiosis cases were defined as the occurrence of the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) diagnosis code 088.82 with no other instances of this code in the 365 days prior. We searched for babesiosis ICD9-CM codes in four file settings: inpatient, outpatient, carrier, and skilled nursing facility.

We estimated annual babesiosis rates by calculating the number of cases recorded per 100,000 beneficiaries in each calendar year, stratified by each beneficiary's state of residence. The national babesiosis rate was the weighted average of these state-level rates. All analyses of CMS data were performed using SAS version 9.2. The trend in babesiosis rates from 2006-2013 was performed using the Cochran-Armitage test for trend and a significance level of $p<0.05$. The county-level heat map was created using R version 3.0.2. In cases where state- level babesiosis occurrence was assumed from third-party data sources, residential population estimates, available from the United States Census Bureau, were used as rate denominators. A national babesiosis rate was calculated as the weighted average of these state-level rates.

Transfusion-transmitted babesiosis risk, TTB units prevented due to testing sensitivity and donor loss due to false positive test results were estimated using formulas that combined data on babesiosis risk among the U.S. elderly by state, blood donation rate, average number of times a donor donates annually, and the sensitivity and specificity of the screening tests. The models were calculated in Mathematica 10.0.

Estimated Donors with Babesiosis = Population x Blood Donation Rate x Babesiosis Rate (1)

True Positive Donors = Estimated Donors with Babesiosis x (1-window period days/365) x sensitivity (2)

Estimated TTB Units Prevented = True Positive Donors x Mean Donations per Year x1 unit per donation (3)

False Positive Donors = (Population x Blood Donation Rate - Estimated Donors with Babesiosis) x (1- specificity) (4)

Assumptions: We made several baseline assumptions about the sensitivities and specificities of different blood screening methods, as well as the number of blood donations received annually. We conducted extensive sensitivity analyses, some of which are reported in the body of the document.

- The two-test, both antibody and NAT method is assumed to have a sensitivity and specificity of 99.98%.
- The single test, antibody only method is assumed to have a sensitivity of 97.00% and a specificity of 99.98%.
- We assume that 5% of a state's population donates blood during a given year.
- Furthermore, we assume that each blood donation is one unit in volume and each donor donates 1.7 times per year.
- We assume for every state that potential donors are persons of 16 years of age and above;
- Sensitivity analyses estimated the changes in TTB risk reduction and units lost due to false positives if these best case assumptions were not met.

Limitations:

- Analyses were based on the administrative databases, and consequently, there is:
    - Difficulty in identifying incident vs. prevalent cases as diagnosis codes do not necessarily represent incident events;
    - Possible misdiagnosis or misreporting of babesiosis diagnosis;
    - Lack of clinical detail for diagnosis code verification and unavailability of information to confirm potentially transfusion-transmitted babesiosis cases;
    - Lack of clinical information to identify *Babesia* species;
    - Test results are generally not available in claims data;
    - State-level results are based on the claims data variable indicating a beneficiary's state of residence, which may not be the state in which the individual was initially infected;
- Asymptomatic babesiosis cases are likely to be undetected in Medicare claims data;
- As elderly are less likely to be exposed and infected with babesiosis due to their reduced mobility as compared to generally younger donor population, the results likely under-estimate number of TTB units prevented and overestimate number of units diverted.
- There is significant uncertainty about the characteristics of any test that may be approved including the sensitivity and specificity of the test, the limit of detection of a NAT, and the time from initial infection to seroconversion which will affect the window period for an antibody test.

Supplementary Table 1: Babesiosis Cases and Rates among Elderly Medicare Beneficiaries, by State, 2006-2013

State*	All Years, 2006-2013	2006	2007	2008	2009	2010	2011	2012	2013
United States Total†	10,301 (5.0)	994 (3.7)	850 (3.2)	1221 (4.7)	1495 (5.9)	1308 (5.2)	1366 (5.4)	1219 (4.7)	1848 (7.1)
Connecticut†	1307 (45.7)	173 (43.9)	166 (43.5)	183 (50.2)	156 (44.3)	122 (35.2)	153 (44.7)	128 (37.7)	226 (67.2)
Massachusetts†	2161 (45.1)	76 (12.7)	128 (21.7)	185 (31.6)	229 (39.3)	230 (39.4)	350 (59.4)	391 (63.4)	572 (89.3)
Rhode Island†	247 (41.9)	34 (45.4)	13 (17.7)	30 (41.4)	33 (46.0)	27 (37.6)	28 (38.1)	21 (28.1)	61 (79.4)
New York	3193 (26.8)	397 (24.5)	269 (17.3)	381 (25.3)	595 (40.7)	535 (37.0)	334 (23.2)	253 (17.6)	429 (29.8)
New Jersey†	980 (13.9)	80 (9.0)	59 (6.7)	98 (11.1)	150 (17.2)	112 (12.8)	169 (19.2)	127 (14.4)	185 (20.8)
Maryland†	312 (7.3)	43 (8.5)	34 (6.7)	60 (11.7)	44 (8.3)	39 (7.3)	24 (4.4)	28 (5.1)	40 (7.0)
New Hampshire†	85 (6.9)	3 (2.0)	4 (2.6)	9 (6.0)	6 (4.0)	10 (6.6)	20 (13.2)	21 (13.3)	12 (7.1)
Maine†	76 (5.6)	8 (4.4)	4 (2.2)	10 (5.7)	6 (3.6)	9 (5.5)	16 (9.8)	10 (6.1)	13 (7.9)
District of Columbia	15 (4.3)	3 (6.8)	1 (2.3)	3 (7.0)	1 (2.3)	-	3 (6.8)	3 (6.7)	1 (2.2)
Virginia†	245 (4.2)	25 (3.3)	30 (4.1)	58 (8.1)	33 (4.6)	28 (3.9)	26 (3.5)	23 (3.0)	22 (2.8)
Minnesota†	104 (3.8)	6 (1.4)	8 (2.0)	8 (2.2)	18 (5.2)	17 (5.2)	28 (9.3)	5 (1.7)	14 (5.0)
Vermont	24 (3.8)	2 (2.6)	2 (2.6)	3 (3.9)	3 (3.9)	1 (1.3)	7 (8.8)	2 (2.5)	4 (4.8)
Pennsylvania†	262 (3.1)	28 (2.4)	18 (1.6)	31 (2.9)	22 (2.2)	34 (3.4)	36 (3.6)	40 (4.0)	53 (5.2)
Delaware	25 (2.9)	2 (2.0)	1 (1.0)	7 (6.6)	4 (3.58)	3 (2.8)	4 (3.6)	1 (0.9)	3 (2.5)
Wisconsin†	111 (2.7)	5 (0.8)	13 (2.4)	14 (2.7)	10 (2.0)	17 (3.6)	23 (4.8)	8 (1.7)	21 (4.4)
Florida†	393 (2.7)	29 (1.5)	33 (1.8)	44 (2.4)	70 (3.8)	50 (2.7)	53 (2.9)	59 (3.3)	55 (3.0)
California†	279 (1.7)	15 (0.8)	10 (0.5)	41 (2.0)	49 (2.4)	31 (1.5)	43 (2.1)	40 (1.9)	50 (2.3)
New Mexico	19 (1.5)	-	3 (1.9)	2 (1.3)	4 (2.5)	3 (1.9)	1 (0.6)	1 (0.6)	5 (3.0)
Colorado†	25 (1.1)	10 (3.5)	3 (1.1)	1 (0.4)	2 (0.7)	3 (1.0)	2 (0.7)	1 (0.3)	3 (0.9)
Kansas	25 (1.0)	2 (0.6)	-	3 (1.0)	8 (2.7)	4 (1.3)	2 (0.7)	2 (0.7)	4 (1.3)
Arizona	29 (0.8)	2 (0.5)	5 (1.3)	5 (1.2)	4 (1.0)	2 (0.5)	5 (1.1)	3 (0.7)	3 (0.6)
Alaska	3 (0.8)	-	-	1 (2.4)	-	-	1 (2.1)	-	1 (1.9)

State*	All Years, 2006-2013	2006	2007	2008	2009	2010	2011	2012	2013
South Dakota	5 (0.6)	2 (1.9)	-	1 (1.0)	-	-	2 (2.0)	-	-
North Dakota	4 (0.6)	-	-	-	1 (1.2)	-	2 (2.5)	-	1 (1.3)
Michigan	45 (0.6)	12 (1.0)	5 (0.5)	3 (0.3)	4 (0.4)	6 (0.7)	5 (0.5)	6 (0.6)	4 (0.4)
Missouri	26 (0.6)	2 (0.3)	2 (0.3)	4 (0.7)	6 (1.0)	3 (0.5)	4 (0.7)	3 (0.5)	2 (0.3)
Oregon	11 (0.5)	1 (0.4)	1 (0.4)	-	2 (0.8)	1 (0.4)	2 (0.8)	-	4 (1.4)
Texas†	65 (0.5)	14 (0.8)	10 (0.6)	10 (0.6)	9 (0.5)	2 (0.1)	7 (0.4)	5 (0.3)	8 (0.5)
Utah	5 (0.4)	1 (0.6)	1 (0.7)	1 (0.7	-	-	-	1 (0.7)	1 (0.7)
Indiana†	21 (0.4)	1 (0.1)	-	3 (0.5)	1 (0.2)	-	-	2 (0.3)	14 (2.2)
Washington	17 (0.4)	1 (0.2)	1 (0.2)	3 (0.6)	3 (0.6)	2 (0.4)	2 (0.4)	2 (0.4)	3 (0.5)
South Carolina	15 (0.4)	1 (0.2)	1 (0.2)	4 (0.9)	1 (0.2)	1 (0.2)	2 (0.4)	2 (0.4)	3 (0.5)
Illinois†	37 (0.4)	-	2 (0.2)	3 (0.2)	-	3 (0.2)	7 (0.6)	4 (0.3)	18 (1.4)
Nevada	5 (0.4)	1 (0.6)	1 (0.6)	-	-	-	-	2 (1.1)	1 (0.5)
North Carolina	26 (0.4)	8 (0.9)	5 (0.6)	2 (0.2)	2 (0.2)	1 (0.1)	-	3 (0.3)	5 (0.5)
Iowa	10 (0.3)	1 (0.3)	1 (0.3)	-	1 (0.3)	1 (0.3)	2 (0.6)	4 (1.1)	-
Georgia	19 (0.3)	-	3 (0.4)	4 (0.6)	1 (0.1)	5 (0.7)	1 (0.1)	3 (0.4)	2 (0.3)
Alabama	11 (0.3)	1 (0.2)	3 (0.6)	-	4 (0.9)	-	-	3 (0.6)	-
Mississippi	6 (0.2)	-	1 (0.3)	-	1 (0.3)	1 (0.3)	1 (0.3)	2 (0.6)	-
West Virginia	4 (0.2)	-	-	-	3 (1.5)	1 (0.5)	-	-	-
Oklahoma	7 (0.2)	-	2 (0.5)	3 (08)	1 (0.3)	-	-	-	1 (0.3)
Montana	2 (0.2)	-	-	-	-	1 (0.9)	-	1 (0.9)	-
Idaho	2 (0.2)	-	-	-	1 (0.8)	-	-	1 (0.8)	-
Nebraska	3 (0.2)	1 (0.5)	-	-	1 (0.5)	1 (0.5)	-	-	-
Ohio	14 (0.2)	2 (0.2)	2 (0.2)	1 (0.1)	4 (0.4)	-	-	3 (0.3)	2 (0.2)
Kentucky	6 (0.2)	1 (0.2)	2 (0.5)	-	-	-	-	2 (0.5)	1 (0.2)
Tennessee	7 (0.2)	1 (0.2)	1 (0.2)	2 (0.4)	1 (0.2)	1 (0.2)	-	1 (0.2)	-
Hawaii	1 (0.1)	-	-	-	-	-	-	1 (1.2)	-
Louisiana	4 (0.1)	-	1 (0.3)	-	1 (0.3)	-	1 (0.3)	1 (0.3)	-
Arkansas	3 (0.1)	-	1 (0.3)	-	-	1 (0.3)	-	1 (0.3)	-
Wyoming	-	-	-	-	-	-	-	-	-

* Includes District of Columbia. States are shown in descending order of babesiosis rate during the 8-year period.

† The trend in babesiosis rates from 2006-2013 is statistically significant according to the Cochran-Armitage test for trend, using a significance level of $p<0.05$.

Supplementary Table 2: Babesiosis Cases and Rates in 31 States, 2011-2013 (CDC)

State	2011-2013				2011			2012			2013		
	Total Number of Cases	Average Annual Cases	Resident Population (in Thousands)	Babesiosis Rate (per 100,000 Residents) [1]	Cases [2]	Resident Population (in Thousands) [2]	Babesiosis Rate (per 100,000 Residents)	Cases [3]	Resident Population (in Thousands) [3]	Babesiosis Rate (per 100,000 Residents)	Cases [4]	Resident Population (in Thousands) [4]	Babesiosis Rate (per 100,000 Residents)
Rhode Island	271	90.3	1053	8.6	73	1,057	6.9	56	1,051	5.3	142	1,050	13.5
Connecticut	486	162.0	3,569	4.5	74	3,527	2.1	123	3,587	3.4	289	3,592	8.0
Massachusetts	894	298.0	6,628	4.5	208	6,631	3.1	261	6,607	4.0	425	6,645	6.4
New York	1206	402.0	16,741	2.4	418	11,146	3.8	254	19,502	1.3	534	19,576	2.7
New Jersey	429	143.0	8,812	1.6	166	8,733	1.9	92	8,835	1.0	171	8,868	1.9
Maine	55	18.3	1,324	1.4	9	1,313	0.7	10	1,329	0.8	36	1,329	2.7
New Hampshire	54	18.0	1,321	1.4	13	1,324	1.0	19	1,318	1.4	22	1,322	1.7
Wisconsin	227	75.7	5,701	1.3	80	5,669	1.4	69	5,710	1.2	78	5,725	1.4
Minnesota	177	59.0	5,339	1.1	73	5,290	1.4	40	5,347	0.7	64	5,380	1.2
Vermont	9	3.0	625	0.5	1	622	0.2	2	627	0.3	6	626	1.0
Delaware	3	1	905	0.1	1	891	0.1	0	908	0	2	917	0.2
North Dakota	2	0.7	680	<0.1	1	654	0.2	0	685	0	1	701	0.1
Maryland	16	5.3	5,821	<0.1	4	5,737	<0.1	3	5,840	<0.1	9	5,885	0.2
South Dakota	1	0.3	826	<0.1	-	-	-	-	-	-	1	834	0.1
Nebraska	1	0.7	1,836	<0.1	0	1,811	0	1	1,842	<0.1	1	1,855	<0.1
Louisiana	2	0.7	4,569	<0.1	0	4,529	0	-	-	-	2	4,602	<0.1
Indiana	1	0.7	6,500	<0.1	0	6,445	0	1	6,516	<0.1	1	6,538	<0.1
California	11	3.7	37,650	<0.1	4	37,267	<0.1	4	37,684	<0.1	3	38,000	<0.1
Oregon	1	0.3	3,875	<0.1	1	3,856	<0.1	0	3,868	0	0	3,900	0
South Carolina	1	0.3	4,664	<0.1	0	4,597	0	-	-	-	1	4,723	<0.1
Alabama	1	0.3	4,784	<0.1	1	4,730	<0.1	0	4,804	0	0	4,818	0
Michigan	2	0.7	9,897	<0.1	0	9,931	0	0	9,877	0	2	9,883	<0.1
Tennessee	1	0.3	6,398	<0.1	1	6,338	<0.1	0	6,400	0	0	6,455	0
Illinois	2	0.7	12,891	<0.1	0	12,944	0	2	12,860	<0.1	-	-	-
Washington	1	0	6,821	<0.1	0	6,746	0	0	6,823	0	1	6,895	<0.1
Texas	1	0	25,635	<0.1	-	-	-	-	-	-	1	26,061	<0.1
Georgia	0	0	9,879	0	0	9,908	0	-	-	-	-	-	-
Utah	0	0	2,833	0	-	-	-	-	-	-	0	2,855	0
West Virginia	0	0	1,846	0	0	1,826	0	-	-	-	0	1,857	0
Montana	0	0	994	0	0	980	0	0	998	0	0	1,005	0
Wyoming	0	0	564	0	0	548	0	0	567	0	0	577	0

[1] The babesiosis rate is the average of the rates from 2011-2013, weighted by the yearly resident population.

Sources:

[2] Ref. 9; http://www.cdc.gov/mmwr/mmwr_nd/

[3] Ref. 10; http://www.cdc.gov/mmwr/mmwr_nd/

[4] Ref. 11; http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6332a6.htm

Supplementary Table 3: TTB Cases and Rates in 22 States, 1979-2014

State	Total Number of Cases	Average Annual Cases*	Average Resident Population (in Thousands)†	TTB Rate (per 100,000 Residents)
Rhode Island	36	1.00	1,013	0.10
Connecticut	23	0.64	3,349	0.02
New York	69	1.92	18,484	0.01
Massachusetts	23	0.64	6,177	0.01
Minnesota	12	0.33	4,708	<0.01
Vermont	1	0.03	598	<0.01
Delaware	1	0.03	745	<0.01
New Jersey	9	0.25	8,126	<0.01
Pennsylvania	12	0.33	12,172	<0.01
New Hampshire	1	0.03	1,161	<0.01
Wisconsin	4	0.11	5,192	<0.01
Maryland	3	0.08	5,083	<0.01
Florida	3	0.08	14,783	<0.01
Ohio	2	0.06	11,159	<0.01
Indiana	1	0.03	5,916	<0.01
Virginia	1	0.03	6,763	<0.01
Georgia	1	0.03	7,598	<0.01
North Carolina	1	0.03	7,618	<0.01
Texas	2	0.06	19,811	<0.01
California	2	0.06	20,261	<0.01
Illinois	1	0.03	12,051	<0.01
Washington	1	0.03	17,047	<0.01

*** Sources:**

Refs. 8 (CDC data); and 14 (Food and Drug Administration Biological Deviation Reports of TTB Babesia cases from January 1, 2010 to December 2, 2014).

† Calculated as the average of the annually estimated resident populations in each state from 2000-2013. Data from the United States Census Bureau:

1979-1989: ://www.census.gov/popest/data/state/asrh/1980s/80s_st_totals.html

1990-1999: ://www.census.gov/popest/data/historical/1990s/state.html

2000-2009: ://www.census.gov/popest/data/historical/2000s/vintage_2009/

2010-2014: http://www.census.gov/popest/data/national/totals/2014/index.html

Supplementary Table 4: Ranking of States Based on Babesiosis Rates

State	Ranking Based on CMS Data 2006-2013	Ranking Based on CMS Estimated donors with Babesiosis	Ranking Based on CDC Data 2011-2013	Ranking based on CDC+FDA TTB rate
Connecticut	1	3	2	2
Massachusetts	2	2	3	4
Rhode Island	3	7	1	1
New York	4	1	4	3
New Jersey	5	4	5	8
Maryland	6	8	14	12
New Hampshire	7	13	7	10
Maine	8	15	6	Not Reported
District of Columbia	9	20	11	Not Reported
Virginia	10	10	Not Reported	16
Minnesota	11	11	9	5
Vermont	12	21	10	6
Pennsylvania	13	9	Not Reported	9
Delaware	14	22	13	7
Wisconsin	15	6	8	11
Florida	16	12	Not Reported	13
California	17	5	24	20
New Mexico	18	23	Not Reported	Not Reported
Colorado	19	16	Not Reported	Not Reported
Kansas	20	24	Not Reported	Not Reported
Arizona	21	17	Not Reported	Not Reported
Alaska	22	34	Not Reported	Not Reported
South Dakota	23	18	12	Not Reported
North Dakota	24	25	15	Not Reported
Michigan	25	35	22	Not Reported
Missouri	26	36	Not Reported	Not Reported
Oregon	27	14	23	Not Reported
Texas	28	26	26	19
Utah	29	19	N/A *	Not Reported
Indiana	30	27	21	15
Washington	31	28	25	22
South Carolina	32	29	19	Not Reported
Illinois	33	30	20	21
Nevada	34	37	Not Reported	Not Reported
North Carolina	35	38	Not Reported	18
Iowa	36	31	Not Reported	Not Reported
Georgia	37	32	Not Reported	17
Alabama	38	39	N/A*	Not Reported
Mississippi	39	33	Not Reported	Not Reported
West Virginia	40	40	N/A*	Not Reported
Oklahoma	41	41	Not Reported	Not Reported
Montana	42	42	N/A*	Not Reported
Idaho	43	43	Not Reported	Not Reported
Nebraska	44	44	18	Not Reported

State	Ranking Based on CMS Data	Ranking Based on CMS Estimated donors with Babesiosis	Ranking Based on CDC Data	Ranking based on CDC+FDA TTB rate
Ohio	45	45	Not Reported	14
Kentucky	46	46	Not Reported	Not Reported
Tennessee	47	47	17	Not Reported
Hawaii	48	48	Not Reported	Not Reported
Louisiana	49	49	16	Not Reported
Arkansas	50	50	Not Reported	Not Reported
Wyoming	N/A*	51	N/A*	Not Reported

* No babesiosis cases were reported in these states.

Supplementary Table 5:
Projected True Positive and False Positive Results by State
Sorted by Babesiosis Rates
Serology Testing in All States, Serology + NAT in Top Five Babesiosis Rate States

State	True Positive Units Results	False Positive Units Results
Total United States	975	4120
Connecticut	106	48
Massachusetts	196	91
Rhode Island	29	15
New York	343	266
New Jersey	78	118
Maryland	26	78
New Hampshire	6	18
Maine	5	18
District Of Columbia	2	9
Virginia	20	107
Minnesota	12	71
Vermont	2	9
Pennsylvania	25	174
Delaware	2	12
Wisconsin	9	77
Florida	33	258
California	39	491
New Mexico	2	27
Colorado	3	67
Kansas	2	37
Arizona	3	85
Alaska	0	9
South Dakota	0	11
North Dakota	0	9
Michigan	3	134

Supplementary Table 5 Continued		
State	True Positive Results	False Positive Results
Missouri	2	80
Oregon	2	52
Texas	8	321
Utah	0	34
Indiana	2	86
Washington	2	90
South Carolina	2	62
Illinois	3	171
Nevada	0	35
North Carolina	2	126
Iowa	0	41
Georgia	2	127
Alabama	2	64
Mississippi	0	39
West Virginia	0	25
Oklahoma	0	49
Montana	0	13
Idaho	0	20
Nebraska	0	24
Ohio	2	155
Kentucky	0	58
Tennessee	0	85
Hawaii	0	18
Louisiana	0	60
Arkansas	0	39
Wyoming	0	7

Supplementary Table 6:
Projected True Positive and False Positive Results by States
Sorted by Estimated Donors with Babesiosis
Serology Testing in All States, Serology + NAT in Top Five Babesiosis Rate States

State	True Positive Units Results	False Positive Units Results
Total United States	975	4120
Connecticut	106	48
Massachusetts	196	91
Rhode Island	29	15
New York	343	266
New Jersey	78	118
Maryland	26	78
New Hampshire	6	18
Maine	5	18
District Of Columbia	2	9
Virginia	20	107
Minnesota	12	71
Vermont	2	9
Pennsylvania	25	174
Delaware	2	12
Wisconsin	9	77
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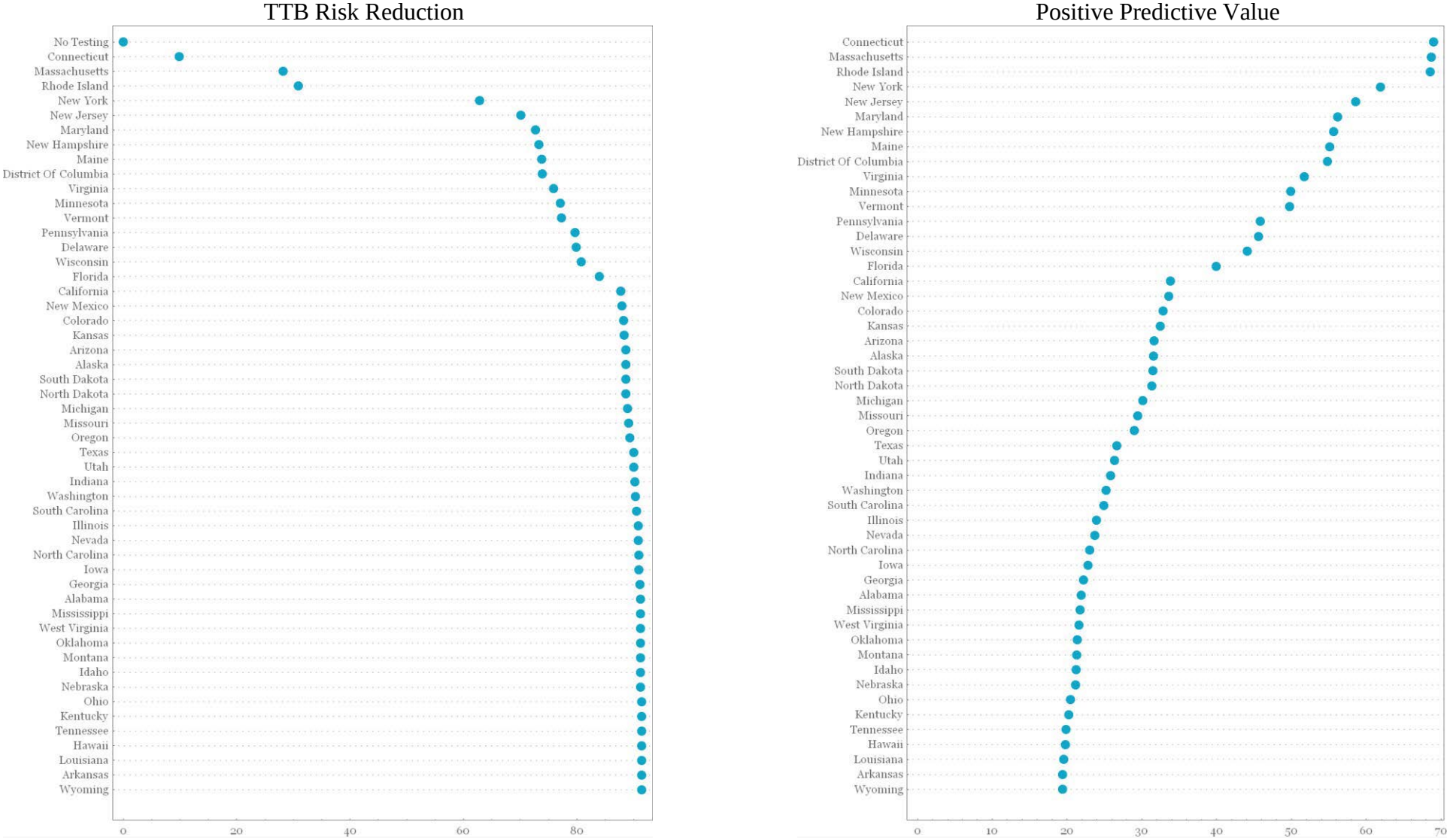
Colorado	3	67
Kansas	2	37
Arizona	3	85
Alaska	0	9
South Dakota	0	11
North Dakota	0	9
Michigan	3	134
Missouri	2	80

Supplementary Table 6 Continued		
State	True Positive Results	False Positive Results
Oregon	2	52
Texas	8	321
Utah	0	34
Indiana	2	86
Washington	2	90
South Carolina	2	62
Illinois	3	171
Nevada	0	35
North Carolina	2	126
Iowa	0	41
Georgia	2	127
Alabama	2	64
Mississippi	0	39
West Virginia	0	25
Oklahoma	0	49
Montana	0	13
Idaho	0	20
Nebraska	0	24
Ohio	2	155
Kentucky	0	58
Tennessee	0	85
Hawaii	0	18
Louisiana	0	60
Arkansas	0	39
Wyoming	0	7

Supplemental Figure 1a: Matrix Plot of Percentage TTB Risk Reduction and PPV of Antibody Testing by State Sorted by Babesiosis Rate



Supplemental Figure 1b: Matrix Plot of Percentage TTB Risk Reduction and PPV of Antibody Testing by State Sorted by Estimated Donors with Babesiosis

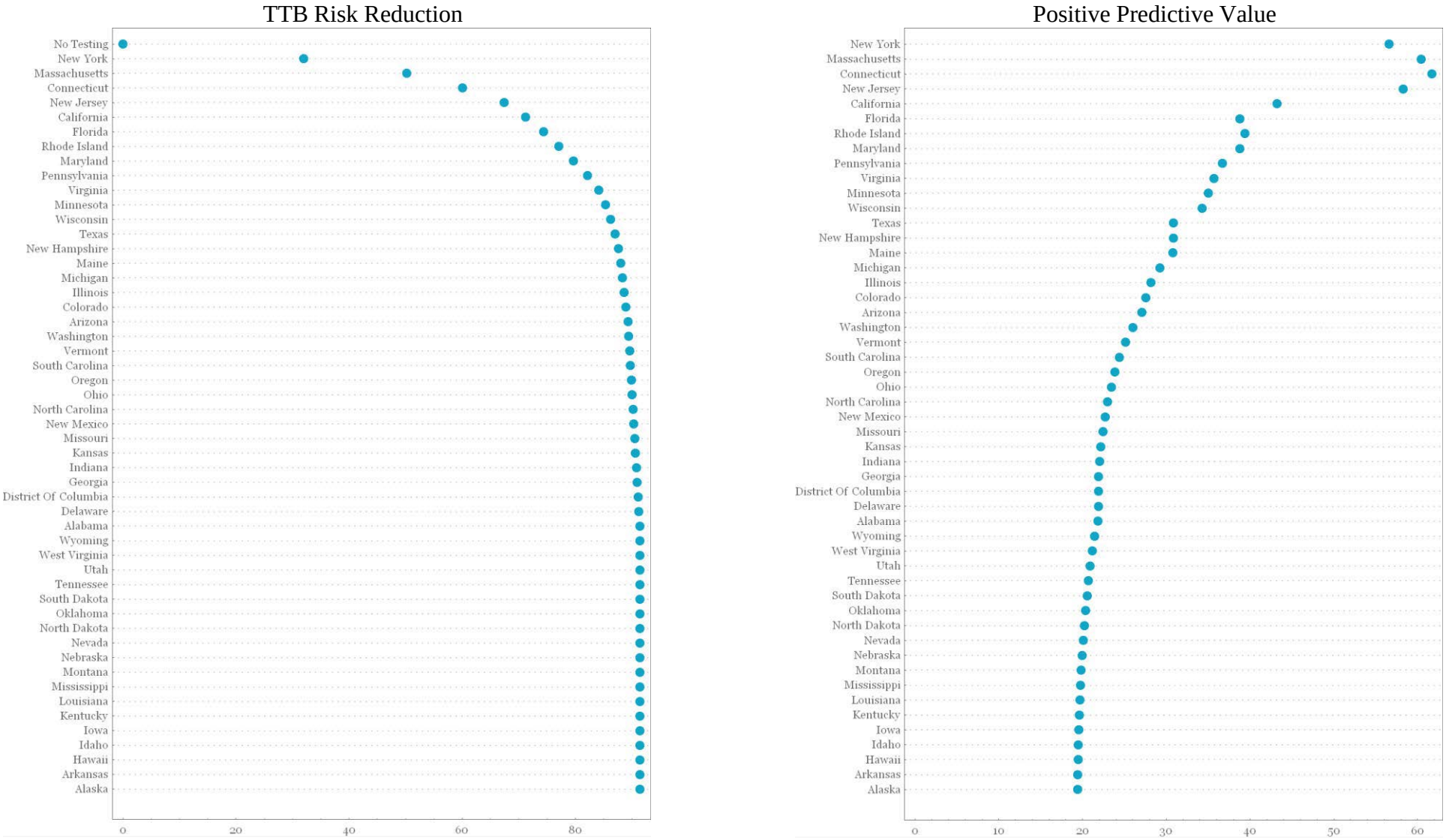
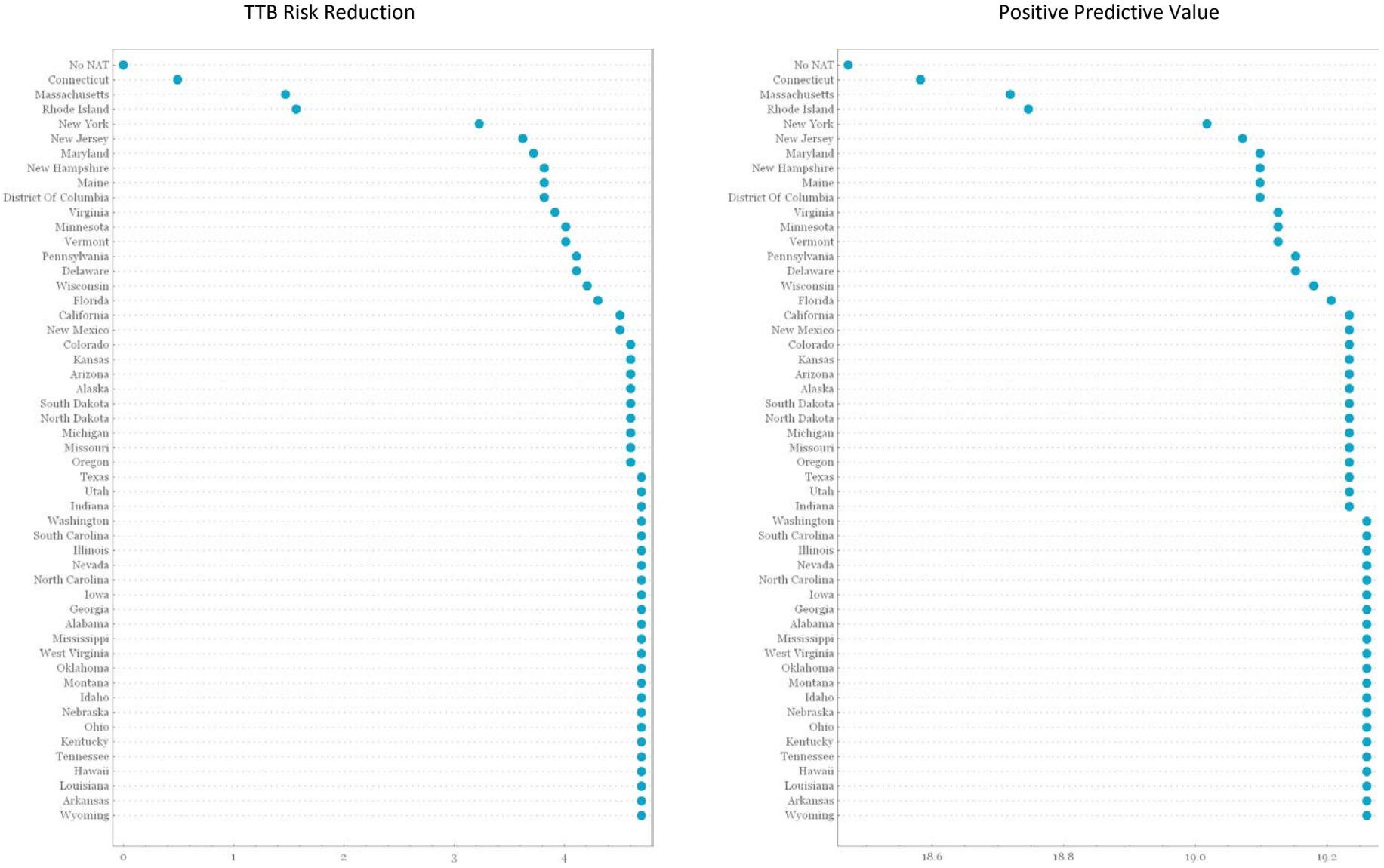


Figure Legend: Dot plots of the percentage of TTB risk reduction and the PPV of year-round national antibody testing of blood units for babesiosis. The top row of the matrix sorts states by the babesiosis rate in the state. The bottom row sorts states by the estimated number of donors with babesiosis. The left column shows the percentage of TTB risk reduction as more states implement antibody testing. The right column shows the PPV as more states implement antibody testing

Supplemental Figure 2a: Matrix Plot of Percentage TTB Risk Reduction and PPV of Antibody Testing Plus NAT by State Sorted by Babesiosis Rate



Supplemental Figure 2b: Matrix Plot of Percentage TTB Risk Reduction and PPV of Antibody Testing Plus NAT by State Sorted by Estimated Donors with Babesiosis

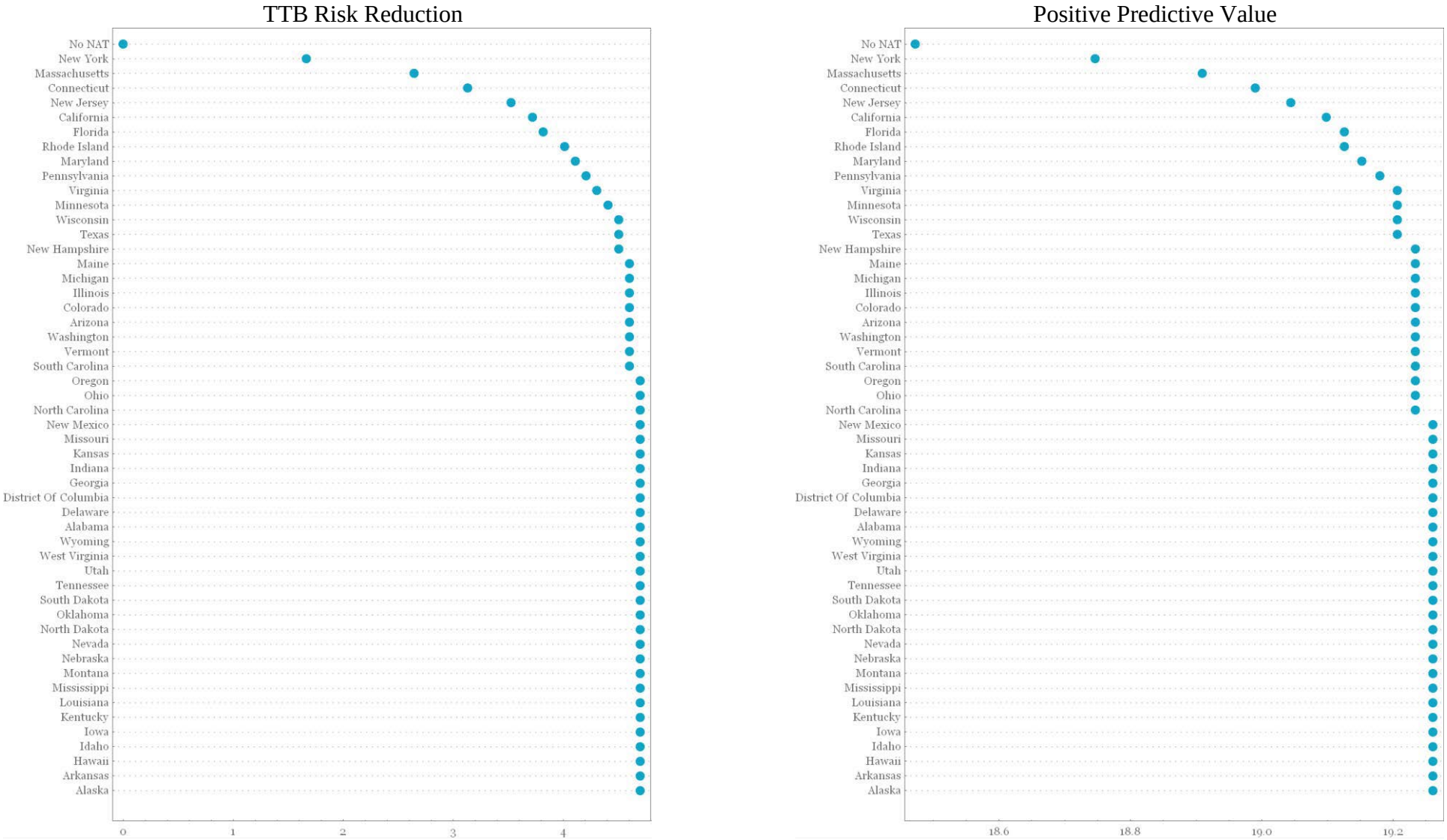


Figure Legend: Dot plots of the percentage of TTB risk reduction and the PPV of year-round NAT in addition to national antibody testing of blood units for babesiosis. The top row of the matrix sorts states by the babesiosis rate in the state. The bottom row sorts states by the estimated number of donors with babesiosis. The left column shows the percentage of TTB risk reduction as more states implement NAT plus antibody testing. The right column shows the PPV as more states implement NAT plus antibody testing

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